Initial Heat Production in Isometric Frog Muscle at 15°C

ALLAN FRASER and FRANCIS D. CARLSON

From The Thomas C. Jenkins Department of Biophysics, The Johns Hopkins University, Baltimore, Maryland 21218

ABSTRACT An infrared radiation-detecting system was used to measure initial heat production in bull frog sartorius muscle at 15°C. Numerous tests with the system showed that thermal artifacts were not noticeable. Many previous measurements with myothermic thermopiles were corroborated with this method. In addition, a cooling phase as large as 0.39 of peak exothermicity was found during and after relaxation. Cooling diminished with both increasing sarcomere length and increasing duration of mechanical activity. No large rapid increase in heat rate accompanied a 0.6 reactivation at the peak of twitch tension. Above rest length, initial heat rate and the heat produced up to the peak of tension decreased nearly proportionally with overlap of myofilaments, while the total twitch initial heat decreased slightly.

INTRODUCTION

Many investigators have measured the heat produced in muscular contractions to determine energy fluxes during the contractions. The first law of thermodynamics requires that the heat plus work produced by a contraction be equal to the sum of the changes of enthalpy associated with the chemical reactions that generate the contraction. Further, the rate of enthalpic change must be equal to heat rate plus work rate at every instant during contraction. Some parts of muscular contractions are rapid and have abrupt changes of heat rate, particularly at higher temperatures. To date, muscle heat production studies (for reviews see Hill, 1965, Mommaerts, 1969, Woledge, 1970) have been limited by uncertainty in time resolution caused by heat flow through inert fascia and water on the muscle surface and into a thermopile. The result is a 5–10 ms uncertainty in time resolution even under fortunate conditions where there is no more than 2 μm of water between the muscle and the thermopile (Hill, 1949 *a*). Thus, it is of interest to improve the time resolution of thermal measurements.

An infrared radiometric system, the "myothermic radiometer," was recently devised to improve the time resolution of myothermometry and to provide an alternative way to measure muscle temperature with no contact

between the sensor and the muscle (Fraser, 1971). The myothermic radiometer was used to perform the isometric initial heat production measurements that are reported here. Studies of heat production at temperatures above zero were motivated by a desire to find out what occurs at temperatures at which frogs move normally, and in expectation of differences in various components of heat production arising from different Q_{10}'s for the various components. The experiments reported here were done with the myothermic radiometer on isometric bull frog sartorius muscles at 15°C. Many features of initial heat production that were previously found by thermopile methods are confirmed by these radiometric studies. In contrast, a marked endothermic phase during and after relaxation was found and explored. Also, it is concluded that rapid increases in heat rate are not a direct result of the release of Ca^{++} from the sarcoplasmic reticulum, and do not necessarily accompany activation.

METHOD

Radiometer

The myothermic radiometer (Fraser, 1971) detects changes in the infrared radiation emitted by a muscle preparation. A muscle's surface is nearly black in the infrared band covered by the 300° K black body emission curve. A 1°mC (millicelsius) temperature change causes about 6 nW of infrared radiation difference per mm^2 of black surface. About half of that radiation is optically collectable. The radiometer monitors radiation from a 7 mm^2 target near the middle of the "inside" surface of the muscle (the surface with only perimesium). Excess physiological saline is blotted from the target surface before measurements are made, and the temporal uncertainty caused by heat flow through the remaining 1–2 μm of wet perimesium is about 0.5 ms.

The radiometer is arranged so that time resolution may be traded off with noise level. In the work reported here, 4 ms and 8 ms single time constant rise characteristics were used with accompanying noise levels of below 1° and 0.5°mC, respectively. An averaging computer was used to increase signal to noise ratios. Random uncertainty in base-line drift followed an approximately time squared dependence, and was about 1.0°mC rms after 1 s on unaveraged traces. No correction was made for heat loss, since the muscles were virtually adiabatic for the few seconds that the longest measurements required. Response time was calibrated by observing the response of the system to abrupt steps of input radiation. A temperature calibrator consisting of a black aluminum block containing a heater was placed so that it occupied the same position as the target area of the muscle. Known temperature increments were generated on the calibrator's surface, and the change in voltage at the output of the radiometer was noted for temperature change calibration.

Tissue Preparation Procedures

A sartorius muscle from a bull frog (*Rana catesbeiana*) was dissected out with half of the pelvic bone attached. A small S-shaped hook was tied directly to the tibial tendon. Dissection and storage were done in cold physiological saline consisting of

114 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$, 1.44 mM NaH_2PO_4, 3.58 mM Na_2HPO_4, 1 g/liter glucose, 5 mg/liter tubocurarine chloride. After dissection, muscles were stored at 4°C for about 5 h and brought to 15°C in about 30 min. Muscles *in situ* were about 40–60 mm long, 8–12 mm wide, and weighed 0.15–0.30 g. Frogs were stored at room temperature and fed meal worms for about a month before being used.

Muscle Mounting

An acrylic muscle mount (Fraser, 1971) was used to position the bull frog sartorius muscle isometrically in the field of view of the detector. The mount provided four pairs of stimulating electrodes, a clamp to hold the split pelvic bone, pins near the target area to further immobilize the muscle, baffling to avoid air currents and maintain water saturation of the air, and a tension transducer. Stimulating electrodes were arranged in bipolar pairs with 1 mm between members and 1 cm between pairs. The immobilizing pins were arranged in two rows 6 mm long and 1 cm apart. The target area of the muscle was set between the rows of pins, and then the lateral fringe fascia was drawn out and held in place by driving it down over the pins. The hook on the tibial tendon was attached to a strain gauge tension transducer whose compliance was 0.2 mm/N. Muscle movement at the target area arising from tetanic stimulation was generally less than 0.25 mm, and at worst less than 0.5 mm. Muscles were mounted and equilibrated to 15°C in physiological saline. The last operations before placing the mounted muscle in the radiometer were draining the chamber and blotting the target surface with a strip of filter paper.

Protocol

The mounted muscle was thermally equilibrated in the radiometer for about 10 min. During this time the minimum stimulus level that produced maximal twitches was found, and about 120% of this level was set for all subsequent stimuli. Stimuli were 0.5 ms square pulses delivered through a transformer. Recorded data consisted of simultaneous muscle tension and temperature change records. Identical stimulation patterns were delivered once per 20 s, and enough serial records were averaged in an averaging computer to produce a smooth averaged record. Muscles were stimulated by this protocol for the equivalent in tension-time integral of no more than 50 twitches over a time of less than 15 min. The individual traces that contributed to the average were monitored on a memory oscilloscope to see that no particularly anomalous traces biased the average and to check that no noticeable time-dependent changes were occurring during the series of traces. Calibration pulses for temperature and tension were electronically added at the beginning of each trace. After data was collected, the sarcomere length of the preparation was measured, and the muscle was cut free of its attachments, blotted, and weighed. The product of tetanic tension, P_o, times muscle length, l, divided by muscle mass, M, was used to find the tetanic stress, $P_o l/M$, developed by each muscle.

Sarcomere Length

The preparation's sarcomere length was measured by optical diffraction immediately after the muscle was removed from the radiometer. A He-Ne laser beam was directed

through the target area of the muscle, and Bragg's law was used to calculate the sarcomere spacing from the observed diffraction pattern. Bull frog muscles gave exceptionally sharp diffraction lines, with line widths of only about 20% of line spacings when the laser beam passed through muscles thicker than 0.5 mm.

Artifact Controls

Calculation and experimentation showed that stimulus heating artifacts were negligible. If the region of tissue near the target is estimated to have stimulus voltage gradients as unreasonably large as 10 V/cm during stimulation, and if the resistivity of the muscles is underestimated at only 10^2 Ω·cm (Katz, 1948), then the heating caused by each stimulus is only 0.1°mC. Some preparations were stimulated until most of the tension had failed. These preparations produced heat records that maintained a level baseline through the early rise of twitch tension. This result could only be obtained if the tension-producing fibers were far from the surface and the joule heating caused by stimulus current flowing near the surface was immeasureably small.

Artifacts of muscle movement are also potentially troublesome in myothermometry. The myothermic radiometer was designed to attain near thermal equilibrium in the neighborhood of the muscle and the mounting procedures were devised to minimize muscle movement. To confirm the utility of these precautions, procedures were devised to search for artifacts.

It could have been that bull frog sartorius muscles were inhomogeneous, or that some regions were not stimulated. Some muscles were stimulated with shocks twice the strength needed to produce maximal twitches. Their heat records appeared to be the same as normally excited tissue, except that fewer stimuli could be given before failure began. A muscle subjected to potassium contractures (Bonner, R. F. 1971. Personal communication of an experiment performed in this laboratory.) showed only phasic tension when placed in physiological saline with up to 100 mM of its Na^+ replaced with K^+, thus indicating that only twitch fibers were present (Kuffler and Vaughan Williams, 1953).

Artifacts could be caused by the muscle moving a region of its surface at a different temperature to and from the field of view of the sensor, or by the field of view of the radiometer extending around a moving lateral edge of the muscle. If they occurred, such artifacts would be as noticeable on the epimesium-covered side of the muscle as on the (thinner) perimesium-covered side that was usually the radiometer target. Heat produced by the muscle itself, on the other hand, would appear only after conduction through the 8 μm of epimesium, a 10 ms delay to 90% response. Experimental records from muscles with their epimesium in view of the detector showed a normal twitch heat pattern with a delay consistent with heat flow through 8 μm of inert surface. In particular, these records showed that the abrupt rise of tension at the beginning of the twitch did not affect the heat record taken from the epimesium-covered side. If the high initial heat rate were a movement artifact, it would have appeared on the epimesium-covered side as well as on the perimesium-covered side.

Muscles were stimulated with a piece of 40 μm thick tissue paper on the surface monitored by the radiometer. The paper was wet and it was small enough to move

with the muscle tissue at and near the target area. Calculation showed a 0.25 s delay to 90% temperature response at the surface of the paper seen by the detector. For the calculation, the wet paper was modeled as a 40 μm thick layer of water. Fig. 1 shows the twitch tension and heat records for the muscle with tissue paper on it, as well as an artists sketch of an earlier heat trace from the same muscle without the tissue paper. This is the muscle that gave the most disturbingly artifact-looking record that was recorded. The heat record on Fig. 1 is as expected for normal muscle heat production coupled with a thermal lag time of about 0.25 s. No effects due to motion can be discerned.

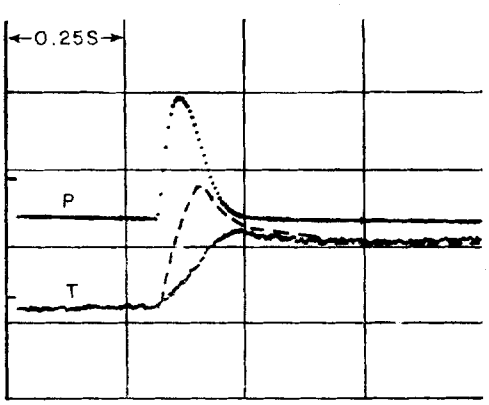

FIGURE 1

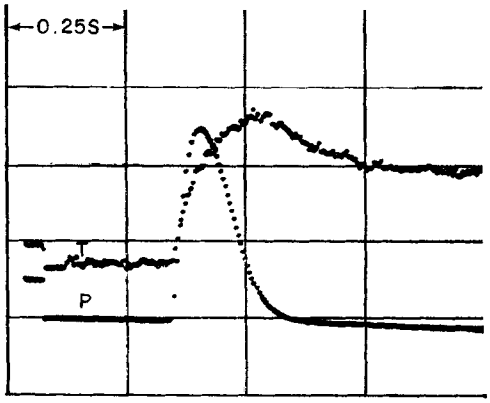

FIGURE 2

FIGURE 1. The twitch isometric tension, *P*, and temperature change *T*, from a muscle with a wet tissue paper on the part of its surface monitored by the radiation detector. The tissue paper was about 40 μm thick and the calculated thermal lag through it was 0.25 s to 90% response. Calibration markers are 20 g tension and 1° mC. Sarcomere length was 2.40 μm. Average of eight traces with 8 ms rise time constant. The dashed line is a drawn-on superposition of the heat trace from the same muscle without the tissue paper on its surface.

FIGURE 2. An isometric twitch tension, *P*, and temperature change, *T*, record from a muscle at nearly rest length. Calibration markers are 20 g tension and 1°mC. Sarcomere length, 2.37 μm. Average of nine traces, 4 ms rise time constant.

RESULTS

Isometric Twitch

Bull frog sartorius muscles at 15°C and near rest length produced heat and tension like that shown in Fig. 2. As the tension, *P*, began rising it was accompanied by a very high rate of temperature change. This initial rate of temperature change, measured as the slope of curve T during the first 20 ms of rising tension, was 0.1°C/s in muscles at rest length. Temperature rates were estimated by examining the digital memory of the transient averager and placing a straight line through the number of samples that corresponded to 20 ms. The beginning of tension and the beginning of heat production were

simultaneous, within a resolution of 2.5 ms. Heat production began at its maximum rate and decreased continuously thereafter until the muscle temperature reached its greatest value during relaxation. During and after relaxation there was a reabsorption of heat (cooling) that will be characterized further below. The final temperature change produced by the twitches in muscles at nearly rest length was between 2.5 and 3.8° mC for all batches of muscles. Cooling appears on Fig. 2 in the region where the slope of curve T is negative, and the final temperature appears as the difference between the initial baseline and the final plateau of curve T.

Different batches of frogs in all seasons uniformly produced data as described above and shown in Fig. 2. Other thermal parameters showed batch variations with scatter comparable with that of the final temperature. Only one muscle out of 28 that were singly stimulated near rest length failed to reabsorb part of its heat during and after relaxation.

Isometric Tetanus

Short isometric tetani at 15°C and near rest muscle length, see Fig. 3, showed a great initial heat rate at the beginning of tension, as the twitches did. A relatively rapid heat production, a labile heat (Aubert, 1956), followed. A constant maintenance heat rate appeared later during tetanus. The time constant of disappearance of the labile heat was 150 ms (±55, SD, $N = 6$) and the maintenance heat rate was 0.027°C/s (±0.008, SD) with stimulation at 40/s.' Heat production continued into relaxation, and was followed by slight cooling during and after late relaxation when the tetani were shorter than about 0.4 s. Resolution to better than 10^{-4}°C with time constants of 4 ms revealed no individual bursts of heat with the stimuli in tetanus.

Twitch Properties Correlated with Sarcomere Length

A group of 25 muscles from one batch of frogs was twitched with the sarcomere lengths of the various muscles spread between 2.45 and 3.08 μm. This

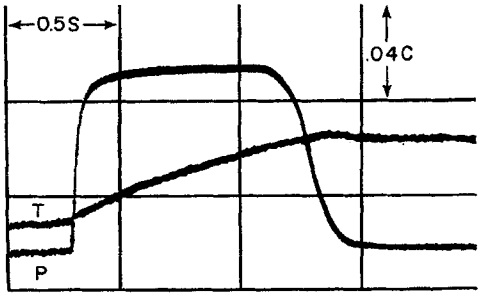

FIGURE 3. Isometric tetanus tension, P, and temperature T, record. Peak tension is 148 g. Unaveraged 8 ms rise time constant. The temperature record shows a burst of heat with the first stimulus, a labile heat, a steady rate of maintenance heat, and slight cooling during and after relaxation.

provided preparations from slightly above rest length to as long as possible without damage by tearing. Correlations appeared between sarcomere length and each of initial heat rate, temperature rise at peak tension, and final temperature rise. Confidence levels in regression coefficients were improved without noticeably altering the values of the regression coefficients by rejecting data from muscles that produced atypically high or low tetanus-twitch tension ratios. The tetanus-twitch tension ratio, 2.4 (±0.5, SD), was itself independent of sarcomere length (regression coefficient, $r = -0.05$). Normalized tetanic tension, $P_o l/M$, correlated well with sarcomere length for all 25 muscles ($r = 0.85$), and nearly as well for the 16 muscles with typical twitch-tetanus ratios ($r = 0.82$). $P_o l/M$ extrapolated to 2.4 μm sarcomere length was 2.7 kg/cm². It therefore appears that those muscles whose data were omitted in the final analysis because of atypical twitch-tetanus tension ratios were unusual specifically in their twitch development. 16 muscles had twitch-tetanus tension ratios within 1 SD of the mean, and they were used in the statistical analysis presented in Table I. Statistics were done

TABLE I

CORRELATIONS BETWEEN THERMAL PARAMETERS AND SARCOMERE LENGTH

	Sample correlation coefficient, r	Value on regression line at 2.52 μm with 90% confidence interval of mean	Value on regression line at 3.02 μm with 90% confidence interval of mean
Initial temperature rate	−0.63	0.075 ± 0.010°C/s	0.046 ± 0.012°C/s
Temperature change at maximum tension	−0.76	2.4 ± 0.22°mC	1.5 ± 0.26°mC
Final temperature change	−0.59	2.63 ± 0.22°mC	2.17 ± 0.23°mC

Data generated from all 16 muscles in one batch of frogs that had typical twitch-tetanus tension ratios.

according to Crow et al. (1960) by correlation analysis and least squared error linear regression. Values of thermal parameters were extracted from the fitted lines at 2.52 μm and 3.02 μm sarcomere lengths.

Near a muscle's center and in the range of sarcomere lengths investigated here, overlap of actin and myosin filaments decreases linearly with increasing sarcomere length. The extrapolated length of no overlap is 3.52 μm (Huxley and Peachey, 1961). The initial rate of temperature, measured during the first 20 ms of tension rise, decreased with muscle length nearly as rapidly as filament overlap did. At an estimated 1 μm of overlap, (2.52 μm sarcomere length) the initial rate was 0.046°C/s. The temperature of the muscle rose to a level at the peak of tension that also showed a dependence on sarcomere length, with values of 2.4°mC and 1.5°mC at 1 μm and 0.5 μm of overlap, respectively. The total temperature rise accompanying twitches was the weakest function of sarcomere length, showing a decrease of only 18% over the same halving of filament overlap.

Fig. 4 shows time-courses of heat and tension produced by a pair of muscles from one frog, one near rest length and one stretched. The peak tension was proportional to filament overlap, but relaxation was much slower with the stretched muscle. All thermal rates were greater near rest length.

Partially Fused Double Twitches

Twitch-tetanus tension ratios are about 0.4 for bull frog sartorius muscles at 15°C. A second stimulation delivered at the time of peak tension in a twitch causes incremental tension and initial tension rate of about the same size as those due to the initial stimulation. Thermal events associated with second

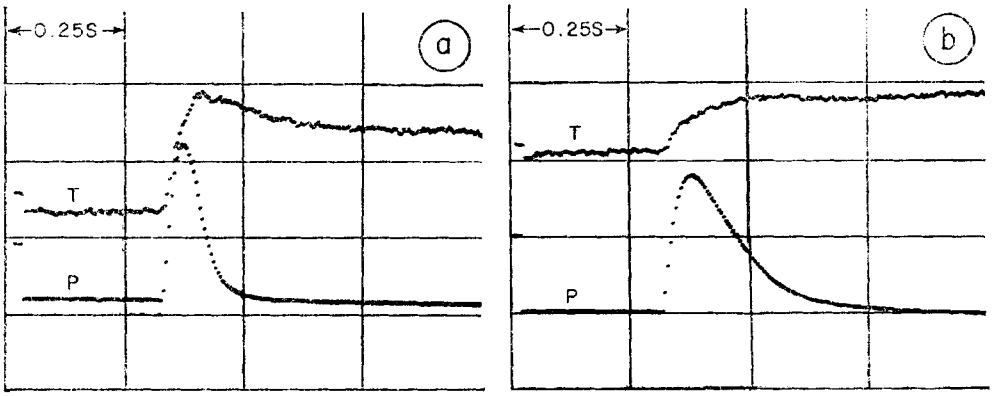

FIGURE 4. Isometric twitches from a pair of sartorius muscles from the same frog. Tension is marked *P* and temperature *T*. Calibration markers are 20 g and 1°mC. The sarcomere length for *a* was 2.28 μm, and for *b* was 3.00 μm. An 8 ms rise time constant was used, and 12 records were averaged for *a* and 9 for *b*. Comparison shows the stretched muscle producing less total heat, heat at peak tension, initial heat rate, twitch tension, and slower relaxation. Cooling virtually disappears with stretch.

stimulations at peak tension and afterwards were investigated. Fig. 5 *a* shows the finding that a second twitch initiated at the peak of tension of a previous one did not produce a sudden increase in heat rate of the same order of magnitude as did stimulation of resting muscle. Fig. 5 *b* shows the finding that second stimuli delivered well into relaxation did produce sudden rate increases of the same order of magnitude as the first stimuli, but always of lesser size. After muscles had completely relaxed, inititial rates of heat for second stimuli became as large as for first stimuli.

13 muscles at sarcomere lengths between 2.4 and 2.9 μm were given second shocks at the peak of twitch tension. Six showed no change in the rate of heat production between the 20 ms time intervals immediately before and after the beginning of increased tension rate caused by the second stimulation; the remaining seven showed slight increases after the second tension rate

increase. For the entire 13, the rate of temperature increase in the 20 ms immediately before the second stimulation was 0.44 (±0.21, SD) of the rate during the 20 ms immediately after the first stimulation, and it rose to 0.54 (±0.17, SD) of the initial rate in the 20 ms after the second stimulation. Therefore, the muscle was reactivated so that it produced a rate of tension nearly as large as after the first stimulus, but that reaction was accompanied by a heat rate change of only 10% of the heat rate caused immediately by the first stimulus.

Cooling Phases

As were presented, isometric twitches near rest length showed cooling during and after relaxation. Further characterization of cooling was done. 10 mus-

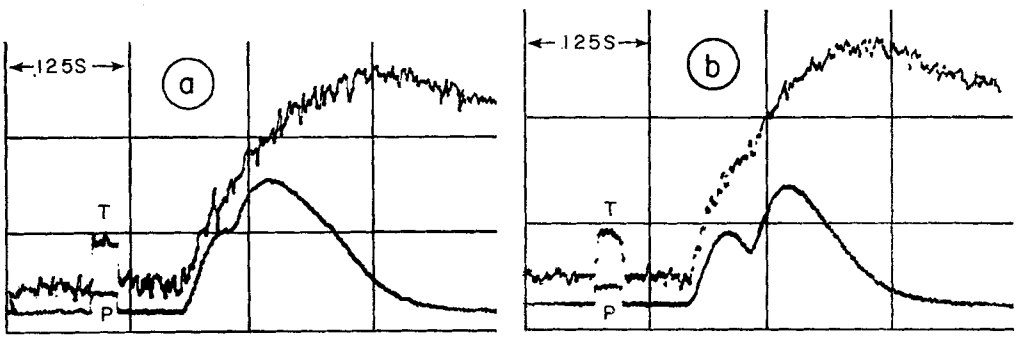

FIGURE 5. Partially fused double twitches. Temperature, T, and tension, P, records from two muscles with calibration markers of 1°mC and 10 g tension. In *a* the second stimulus was given at the peak of tension of the first twitch and no burst of heat production accompanied it; 4 ms rise time constant, average of 10, 2.45-μm sarcomeres. In *b* the second stimulus was given during relaxation and caused a burst of heat; 4 ms rise time constant, average of 10, 2.48-μm sarcomeres.

cles from one batch of frogs were stimulated at approximately rest length (from 2.25 to 2.60 μm sarcomere lengths). The set produced a half time of mechanical relaxation from peak tension of 65 ms (±15, SD) and reached its peak temperature also at 65 ms (±34, SD) after the peak of tension. Cooling continued for a few tenths of a second and required 99 ms (±40, SD) for the first half of total cooling to occur. The fractional amount of the peak heat produced that was reabsorbed was 0.39 (±0.09, SD).

Stretching muscles above rest length diminished the cooling observed during and after relaxation. The effect was progressive with increase in muscle length as far as muscles were stretched. Fig. 4 shows a great diminution of cooling in the stretched muscle. Five records were produced by muscles stretched to sarcomere lengths between 2.9 and 3.2 μm. These muscles were obtained from frogs in the same batch of frogs as the 10 used above at nearly

rest length. They showed a fractional cooling of 0.08 (±0.06, SD) of peak heat production, and finished at a net temperature rise of 3.2°mC (±0.7, SD). Many stretched muscles produced no cooling. This lack of cooling or insignificant cooling near 50% filament overlap is in marked contrast to the 0.39 fractional cooling found near rest length. The set of stretched muscles had a half time of relaxation of 95 ms (±15, SD). Cooling, if it occurred, began at about the half point of mechanical relaxation, as was the case at rest length.

Short tetani at rest length also produced cooling during relaxation, but to a lesser extent than twitches did. Longer tetani showed a reduced or nonexistent cooling phase with relaxation. The limiting duration after which there was no cooling was from 0.4 to 1 s for various muscles. Fig. 6 *a* was

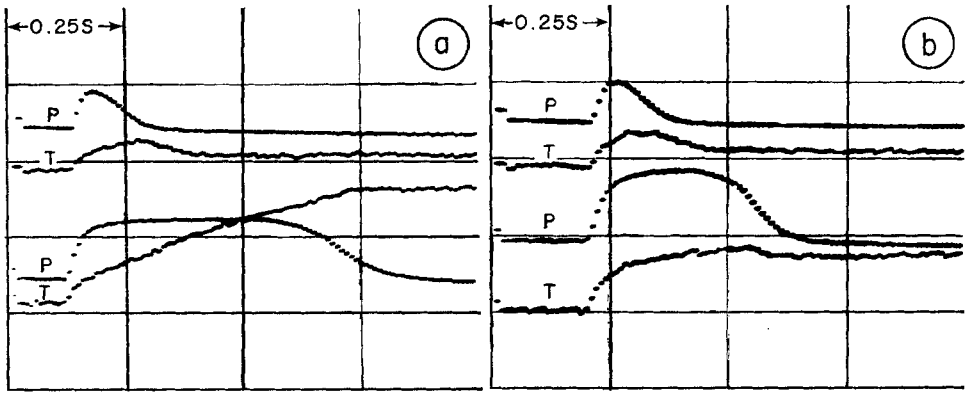

FIGURE 6. Twitch and short tentanus records. Each record was made by alternately twitching and tetanizing with 20 s between contractions. Temperature records, *T*, calibrated with 1° mC markers at left and tension records, *P*, calibrated with 20-g markers at left. Each twitch and tetanus average of four with 8 ms rise time constant. 2.35 μm sarcomere length with *a* and 2.38 μm with *b*. With longer stimulation cooling diminishes to zero and relaxation takes more time.

produced by alternately twitching and producing 0.2 s tetani in a muscle. Cooling was present with both contractions, but it was smaller with the tetanus. The same protocol was used to produce Fig. 6 *b*, but 0.4 s tetani were used. In this case, the tetanic cooling disappeared entirely. It is noteworthy that the longer tetanus in Fig. 6 *b* relaxed more slowly than did the one in Fig. 6 *a*.

DISCUSSION

A General Agreement

The data presented above is in agreement with most aspects of myothermic data obtained from thermopile experimentation. With the exception of the

late cooling phase, the time-course of isometric twitch heat production in bull frog sartorius muscle at 15°C is similar to that found at 0°C in frog and toad muscles (Hill, 1949 *a*, *b*). Both measurement techniques showed a sudden increase in heat rate accompanying the initiation of tension, and then a continuously declining rate until after the peak of tension. Total twitch initial temperature changes in bull frog muscles were from 2.5° to 4°mC, as compared to 2.7°mC (±0.9) found by Gibbs et al. (1966) at 16°–23°C in *Rana pipiens* sartorius muscles. Radiometric measurements also showed a heat production during slow relaxation from tetani of long duration or twitches in preparations with long sarcomere lengths, (see Figs. 4 and 6). Increased heat production during relaxation appeared with slight stretch or short tetanus as a diminution of cooling during relaxation and, with greater stretch or long tetanus as net exothermicity during relaxation. This heat production during the maintained tension of slow relaxation may correspond to the "positive feedback" process reported by Hill (1964) or to the "tension-time" energy requirements discussed by Mommaerts (1969).

Tetanus heat production showed the expected initial burst, labile heat, and maintenance heat when measured with the radiometer. Aubert (1956, p. 159) presented a mean maintenance heat rate for frog muscle of 113 g·cm/g·s at 0°C, and 547 g·cm/g·s at 10°C. The rate reported in this paper at 15°C is 1.0×10^3 g·cm/g·s if 0.86 calorie/degree (cal/deg) is taken to be the specific heat of frog muscle (Hill, 1949 *b*). In the same table, Aubert presented time constants for labile heat of 1.12 s at 0°C and 0.39 s at 10°C. The time constant of labile heat production from the data presented here is 0.15 s at 15°C. Tetanus heat parameters are therefore consistent between Aubert's work and that presented here. No great importance can be attributed to the consistency, however, because of the dependence of both parameters on stimulus rate and previous muscle activity, as well as possible species differences.

Heat Production Immediately after Stimuli

The above data show that a second stimulus delivered at the peak of tension in a twitch causes no dramatic increase in heat rate, as a first stimulus does. Likewise, the individual stimuli in maintained tetani produce no individual thermal changes. Also, stimuli delivered at the time of half relaxation after tetani do not cause any immediate increases in heat rate (Fraser, 1972). These findings suggest a modification of previous ideas about activation heat.

The stimuli delivered during declining tension after a tetanus and at the peak of twitch tension each would increase the active state in the muscle from about one-half to about one (Jewell and Wilkie, 1960). Hill's analysis (1949 *b*) in amphibian muscles at 0°C shows that only activation heat is a major heat source shortly after first stimuli. We might expect an activation

heat after subsequent reactivations, and they do not appear. Therefore, if activation heat is the dominant heat source shortly after activation, as in Hill's analysis, we must conclude that reactivation of partly relaxed muscles need not be accompanied by an "activation heat." Therefore, activation heat must correspond to some other process than activation itself.

Homsher et al. (1972) and Smith (1972) show a "tension-dependent" or "actomyosin-dependent" heat that would accompany activation heat production in the first 20 ms of a twitch at room temperature. This viewpoint is supported by the regression of initial heat rate vs. sarcomere length (Table I), which extrapolates to a finite activation heat rate at 3.52 μm and shows an increasing tension or actomyosin-dependent heat with shorter sarcomere lengths. The double twitch and twitch after tetanus experiments produced tension rates for the last stimuli that were about as large as those caused by the first stimuli (Fig. 5). If the heat rates in the first 20 ms of tension development after stimuli are composed of activation plus tension or actomyosin-related heat rates, then we must again conclude that those component rates do not have the same behavior in partly active muscle as they do in resting muscle.

The second point is that the release of Ca^{++} from the sarcoplasmic reticulum is not a major exothermic event. A pulse of Ca^{++} of nearly the same size as the first one is abruptly released with a second stimulus delayed by 50 ms at 12°C in toad muscle (Jobsis and O'Connor, 1966). The double twitches reported here were at 15°C and at or nearly at 50 ms delay between stimuli. The absence of a second burst of heat production requires that the release of Ca^{++} from the sarcoplasmic reticulum and the dilution of the released ions be of slight thermal importance.

Homsher et al. (1972) and Smith (1972) argue that activation heat is probably a reflection of the activity of the Ca^{++} pump in the sarcoplasmic reticulum. The time-course of activation heat in twitches nearly follows the time-course of free sarcoplasmic Ca^{++} (Jobsis and O'Connor, 1966; Ashley and Ridgeway, 1970). Second stimuli delayed 50 ms at 12°C in toad muscles produce a large Ca^{++} release (Jobsis and O'Connor, 1966). Thus, releases are expected with the second stimuli in the doubly twitched muscles reported here. Also, the individual stimuli in tetani are expected to release individual quanta of Ca^{++}, and the twitch delivered during tetanic relaxation is expected to be preceded by a large Ca^{++} release. None of these stimuli produce sudden heat rate increases that could be ascribed to heat immediately associated with Ca^{++} release. The idea that activation heat reflects immediate consequences of Ca^{++} release is therefore not supported by these findings; the idea that activation heat derives from the Ca^{++} pump is supported if one further idea is introduced. That is: the heat-producing binding or pumping sites in the sarcoplasmic reticulum were already occupied by Ca^{++} released by earlier stimuli when the later stimuli were delivered.

Endothermicity

A cooling phase appears in these measurements on *R. catesbeiana* sartorius muscles at 15°C. It begins during relaxation in isometric twitches and short tetani, and continues until well after the end of relaxation. Efforts to show that the cooling was an artifact failed. Published time-courses of muscle heat production at 0°C frequently reveal a cooling phase. Unfortunately, technical limitations prevent performing experiments at 0°C with the myothermic radiometer. Hill (1949 *a*, Fig. 3) shows a record from a toad sartorius muscle at 0°C in which the unloaded muscle shortened about 3 mm, and, while doing so, reabsorbed about 8% of the heat that it had evolved. Hill, (1964, Fig. 2) again showed cooling in toad muscles at 0°C; it was about 3% after an isometric twitch, and about 5% after a quickly released isometric twitch. Recently, Smith (1972, Fig. 1) showed about 5% cooling after isometric twitches below rest length in frog semitendinosus muscle at 0°C. Hill (1961) studied the "negative-delayed heat," an endothermic phase after short tetani. He found cooling in the 4% range after short tetani. The cooling evolved over seconds at both 0° and 16.7°C. Longer tetanic duration abolished cooling.

The posttetanic cooling that was found with the myothermic radiometer was like that found by Hill in that it decreased and disappeared with longer tetani. However, the shortest tetanic durations that produced no cooling in this research were less, only 0.4 s. Total quantities of cooling were about 1 mcal/g with both techniques, but the endothermic phase observed by Hill required several seconds to evolve, while that found here was always complete within a few tenths of a second.

Most events that occur during relaxation in frog muscle have unknown enthalpies. One known possibility for the source of cooling during and after relaxation is the Lohmann reaction, wherein ADP is rephosphorylated to ATP by the hydrolysis of creatine phosphate (PCr). That reaction was estimated to be endothermic under in vivo conditions, at 0°C with an enthalpic change of −3.7 kcal/mol (Woledge, 1970). Gilbert et al. (1971) demonstrated a breakdown of PCr during relaxation in the range of 10^{-7} to 10^{-6} mol/g after tetani at 0°C in frog muscle. The coupling of PCr utilization during relaxation to ATP formation via the Lohmann reaction would absorb about 1 mcal/g of heat. Gilbert et al. (1971) show no increase in ATP in muscle after activity (in fact, they show a slight decrease), so the newly formed ATP must be consumed by reactions that are themselves endothermic or only slightly exothermic for a net cooling to be observed. Tension-time and feedback heats, thermoelastic heat, and degraded internal work all are very slight during late relaxation, and would therefore provide very little heat to cancel any endothermic processes. Fluorodinitrobenzene (FDNB) blocks

the Lohmann reaction, and should therefore abolish the cooling if it is caused by that reaction. Such was found to be the case: the poisoned muscle produced total heat about equal to peak heat of unpoisoned muscles, and no cooling. FDNB-poisoned muscles relax more slowly than normal muscles, complicating interpretation of such experiments.

Huxley and Simmons (1970) show that inhomogeneous length changes occur along the length of muscle fibers during relaxation, even including shortening of some sarcomeres. The myothermic radiometer requires viewing sartorius muscles in one localized zone which is at or near the end plate region. Perhaps some spatial inhomogeneity is noted with this technique that would be averaged out with thermopile methods.

Disappearance of the endothermic phase with stretch in twitches and with increased duration of tetani has a plausible explanation within present knowledge about muscle heat production. Stretch produces a pronounced decrease in the rate of relaxation in bull frog sartorius muscles at 15°C (Figs. 4, 6) and in other amphibian muscles (Mittenthal and Carlson, 1971; Jewell and Wilkie, 1960). The difference between the final temperature and the temperature at peak tension (Table I) is the heat evolved during relaxation. This quantity increases with stretch. Since less internal work is dissipated in twitches in stretched muscles, it follows that the greater heats produced in relaxation must have come from active metabolism. Longer tetani also showed decreased relaxation rates by a factor of about 2.5 times the twitch relaxation rates (Mittenthal and Carlson, 1971; and Fig. 6). The prolonged tension during the slower tetanic relaxation was also accompanied by more heat production. Heat productions during slow relaxation from tetani or twitches in stretched muscles must involve some metabolism and may be the feedback or tension-time heats. Hill (1964) estimated the feedback heat in an isometric twitch at rest length at 0°C to be 15–20% of the total twitch initial heat. This amounts to about 0.5 mcal/g. For results presented in this paper, peak cooling was about 1.5 mcal/g, and feedbcak heat processes during the very slow relaxations after long tetani could account for the reduced amount of cooling observed.

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